FISHERY RESEARCH

PARENTAGE BASED TAGGING OF SNAKE RIVER HATCHERY STEELHEAD AND CHINOOK SALMON

ANNUAL PROGRESS REPORT
July 1, 2011 — June 30, 2012

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IDFG Report Number 12-09
June 2012

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Project Progress Report

2011 Annual Report

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Division of Fish and Wildlife
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Project Number 2010-031-00
Contract Number 53238

IDFG Report Number 12-09
June 2012

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ABSTRACT

This report summarizes the progress in the continuing development and evaluation of a new genetic technology called Parentage Based Tagging (PBT), which can serve as a versatile tool for the genetic tagging steelhead and Chinook salmon in the Snake River basin. While PBT is potentially a more economical and efficient technique for tagging fish than coded wire tags (CWT); it also has the capability to address aspects of hatchery reform, salmonid life history, harvest patterns, and trait heritability. This report summarizes three objectives for this fiscal year that focused on the feasibility of developing and implementing PBT in the Snake River basin: Objective 1) annual sampling of hatchery broodstock, Objective 2) creation of genetic parental databases, and Objective 3) utilization of PBT to provide parentage assignments for hatchery fish of unknown origin. This project continues to sample and inventory nearly 100% of hatchery broodstock (Objective 1) for steelhead (~5,500 individuals annually) and spring/summer Chinook salmon (~8,000 individuals annually). In close collaboration with the Columbia River Inter-Tribal Fisheries Commission (CRITFC), we have used the PBT SNPs identified for each species to genotype nearly 100% of the steelhead and spring/summer Chinook salmon broodstocks sampled in the Snake River basin from spawn year (SY) 2010 and 2011 (Objective 2). In addition, summary data for Chinook broodstocks from SY2008 and SY2009 are presented. We then use the data generated from the broodstock baselines to provide comparisons between PBT-assignments and known-origin CWT samples, to determine the origin of hatchery strays and to identify the source of hatchery kelts (Objective 3). Results, thus far, indicate that annual sampling, inventorying, and genotyping of all steelhead and spring/summer Chinook salmon broodstock in the Snake River basin is feasible and that the SNP sets identified for PBT are sufficient for accurate assignment of offspring to brood year and hatchery stock, thereby allowing an unprecedented ability to mark millions of Snake River smolts and an opportunity to address future objectives of parentage-based management. Currently, we are beginning the process of demonstrating the utility and versatility of using PBT-tagged stocks for conducting hatchery evaluations and reform, refining estimates of in-river harvest rates, and monitoring hatchery straying in natural spawning areas.

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INTRODUCTION

For over 40 years, researchers and managers in the Columbia River basin have used coded wire tags (CWTs) to monitor and assess harvest patterns and survival rates of salmon and steelhead in the Columbia River basin (Johnson 2004). Recovery of CWTs are one of the primary tools used by managers in Oregon, Washington, and Idaho to estimate the number of hatchery Chinook salmon *Oncorhynchus tshawytscha* and steelhead *O. mykiss* contributing to in-state and out-of-state fisheries and to estimate harvest of individual hatchery stocks.

Despite the predominance of CWT technology in addressing management concerns, it has several limitations. The process of physically tagging tens of thousands of juveniles from different hatchery stocks is logistically difficult, labor intensive, costly, and potentially increases physiological stress to the juveniles just prior to their release for downstream migration. All of these restrictions ultimately limit the total number of juveniles that are tagged each year, which in turn limits the number of CWT recoveries. The resulting small sample sizes greatly reduce statistical power to estimate stock contributions because the precision of these estimates are directly related to the number of CWTs recovered in fisheries or escapements (Hankin et al. 2009).

Parentage-based genetic tagging (described in Anderson and Garza [2005]), a technological alternative to CWT, would eliminate the problem of small sample sizes. Parentage-based tagging (PBT) involves annual sampling and genotyping of hatchery broodstock, creating a database of parental genotypes. Progeny from any of these parents (collected either as juveniles or adults), if genotyped, could be assigned back to their parents, thus identifying their hatchery of origin and their exact brood year. The exceptional advantage that PBT has over CWT technology is increased sample size. By genotyping all parental broodstock, every juvenile is genetically "tagged."

While theoretically appealing (Anderson and Garza 2005; 2006), PBT technology still needs to be empirically tested and validated. Over the last several years, several committees and science review groups have recommended that two or more large-scale evaluations of the technology be performed (PFMC 2008; PSC 2008; ISRP/ISAB 2009).

Given these recent advancements, this project constructs the first PBT genetic baselines for steelhead and Chinook salmon hatcheries in the Snake River basin. It also addresses both current and future objectives in creating PBT baselines within the Snake River basin that can be used for monitoring harvest of hatchery stocks but, also for addressing additional issues, such as the origin of hatchery strays and steelhead kelts, effectiveness of hatchery mitigation programs, broodstock integration, and relative reproductive success of hatchery fish.

OBJECTIVES

For this fiscal year, the Snake River PBT project includes several objectives as follows:

Objective 1: Genetic sampling of hatchery Chinook salmon and steelhead broodstock

Completion of this objective demonstrates the feasibility of sampling and inventorying all hatchery broodstock each year for steelhead and Chinook salmon and recording accurate biological information for every fish.

Objective 2: Creation of parental databases for Snake River hatcheries

Completion of this objective demonstrates the ability to genotype all sampled broodstock and to create a database of parental genotypes for each spawn year of steelhead and spring/summer Chinook salmon.

Objective 3: Utilization of PBT methods to provide accurate parental assignments

We demonstrate the application of this technology through “back end” projects that demonstrate the versatility of PBT. This includes: 1) a paired CWT and PBT recovery experiment as part of existing Lower Snake River Compensation Plan (LSRCP) hatchery evaluations in Idaho, 2) an assessment of the origin of straying hatchery steelhead in the Deschutes River at Sherars Falls, 3) identification of hatchery-origin steelhead kelts out-migrating past Lower Granite Dam. Results from two additional projects that are underway will be included in the next report: 1) an assessment of the stock composition of hatchery Snake River steelhead harvested in the main-stem (Zone 6) Columbia River fishery and 2) run reconstruction of hatchery steelhead at Lower Granite Dam.

REPORT STRUCTURE

This report is divided into three sections, one for each of the objectives for this fiscal year. The first section reports on sampling efforts. The second section summarizes genetic data from the most recently genotyped broodstocks. The third section provides an overview of current implementation and results of PBT projects.

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SECTION 1: ANNUAL SAMPLING OF HATCHERY STEELHEAD AND SPRING/SUMMER CHINOOK SALMON BROODSTOCK IN THE SNAKE RIVER BASIN

Introduction

The implementation of PBT methods requires a complete sampling of broodstock from all hatcheries contributing to the production of steelhead and Chinook (Figure 1). This objective addresses the feasibility to annually sample tissue from 100% of the hatchery broodstock for spring/summer Chinook and steelhead in the Snake River basin.

Methods

The overall goal is to obtain high quality tissue samples and accurate biological data from every adult that contributes to spawning. Biological data includes species, sex, hatchery/stock, date sampled/spawned, tag information, and markings. Most hatcheries also record length and cross information. Tissue samples are collected in the form of fin tissue or operculum punches, stored in 2 ml vials of 100% non-denatured ethanol, and shipped to the IDFG genetics lab in Eagle, Idaho. Care is taken to avoid contamination during sampling by rinsing scissors or hole-punch tools in water or ethanol and wiping with a paper towel in between each tissue sample.

An alternative dry-storage method is also being explored that would eliminate the use of ethanol. This method requires the tissue to be placed on or between sheets of absorptive chromatography paper. Tissue mounted on the paper, once completely dry, has been shown to yield high quality DNA while reducing processing time of samples in the lab (LaHood et al. 2008). Our future collections of broodstock may transition to this methodology if we verify a high genotyping success rate of samples collected using chromatography paper from our targeted broodstocks.

Each sample is labeled with a field ID#, which is used to track the samples until they arrive at the lab, at which time they are given a standardized lab database code. The associated data is reviewed at the lab to ensure accurate information was recorded for every fish sampled. Any discrepancies that are discovered are solved via correspondence with the hatchery employee in charge of recording data. Samples from broodstock whose eggs were culled are not genotyped because they do not produce offspring.

Once the samples are extracted and genotyped, genetic data are recorded into a Progeny database and stored with collection information and individual fish data. Due to the scope of this project, this database was recently created to manage, organize, and track physical tissue samples along with their associated DNA extractions and genotypes. Progeny allows genetic data to be exported along with individual fish data in a variety of formats, which has proven to be essential for the transfer of data between the collaborating IDFG and CRITFC laboratories.

Results

For FY2011-2012, we have collected and inventoried approximately 11,200 genetic samples from the steelhead broodstock (Table 1) and approximately 16,400 samples (Table 2) from Chinook salmon broodstock spawned in the Snake River basin during SY2010 and SY2011. Most hatcheries provided biological information on all fish sampled (sex and length). Some hatcheries provided individual cross information.

Discussion

We continue to demonstrate the feasibility of large-scale sampling and inventorying of thousands of broodstock fish each year. The annual completion of this objective lays the foundation for the use of PBT baselines in the Snake River basins.

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SECTION 2: CREATION OF GENETIC DATABASES FOR BROODSTOCKS OF STEELHEAD AND SPRING/SUMMER CHINOOK SALMON IN THE SNAKE RIVER BASIN

This section presents summary information for the genetic data collected from steelhead broodstocks in SY2010 and SY2011 and Chinook salmon broodstocks in SY2008, SY2009, and SY2010. The Chinook SY2011 broodstock has been genotyped but summary information will be presented in the next annual report for fiscal year 2012-2013.

Introduction

A set of PBT SNPs was identified for steelhead and Chinook salmon, and it was demonstrated that the selected SNPs would provide sufficient resolving power (Steele et al. 2011). These markers were used to genotype broodstock samples collected in 2010 and 2011 (Table 1 and 2).

During the second year of this project (FY2011), IDFG and CRITFC labs extracted and genotyped all sampled for steelhead and Chinook salmon broodstocks (~14,000 IDFG, ~14,000 CRITFC = ~28,000 total samples). By the next contract year (FY2012) the backlog of samples collected in all previous spawn years will have been genotyped and only samples collected in SY2012 will need to be genotyped.

Creation of these parental genetic databases establishes an unprecedented ability to mark millions of Snake River smolts and an opportunity to address a variety of parentage-based research management objectives.

Methods

Laboratory protocol

Genomic DNA extraction and amplification and SNP genotyping using multiplex 5'-nuclease reactions followed the methods described in Matala et al. (2011). DNA was extracted using the Nexttec Genomic DNA Isolation Kit from XpressBio (Thurmont, Maryland) or Qiagen DNeasy (Valencia, California). Prior to DNA amplification of SNP loci using primer-probe sets (fluorescent tags), an initial polymerase chain reaction (PCR) "pre-amp" was implemented using whole genomic DNA to jumpstart SNP amplification via increased copy number of target DNA regions. The PCR conditions for the pre-amp step were as follows: an initial mixing step of 95°C for 15 min, followed by 14 cycles of 95°C for 15 seconds and 60°C for four minutes, ending with a final 4°C dissociation step. For steelhead, all individuals were genotyped at 95 SNPs and a Y-specific allelic discrimination assay that differentiates sex. For Chinook salmon, all individuals were genotyped at 95 SNPs (including one mtDNA SNP) and a Y-specific allelic discrimination assay that differentiates sex. Genotyping was performed using Fluidigm 96.96 Dynamic Array IFCs (chips). For each genotyping run, 96 samples (including an extraction negative control, a PCR negative control, and a PCR positive control) and 96 TaqMan SNP assays were either hand-pipetted or auto-pipetted onto the 96.96 chips. Sample cocktail and SNP assay cocktail recipes are available by request from mike.ackerman@idfg.idaho.gov. Each 96.96 chip was pressurized to load the DNA and SNP assays into the array using a Fluidigm IFC Controller HX. SNP amplification on the 96.96 chips were performed using either an Eppendorf Stand-Alone Thermal Cycler (protocol: thermal mixing step of 50°C for 2 min, 70°C for 30 min, and 25°C for 10 min, a hot-start step of 50°C for 2 min and 95°C for 10 min, followed by 50 cycles of 95°C for 15 sec and 60°C for 60 sec, and a final cool down step of 25°C for 10 min) or a Fluidigm FC1 Fast-cycler (protocol: thermal mixing step of 70°C for 30 min and 25°C for 10 min, a hot-start

step of 95°C for 60 sec, followed by 50 cycles of 95°C for 5 sec and 25°C for 25 sec, and a final cool down step of 25°C for 10 min). Chips were imaged on a Fluidigm EP1 system and analyzed and scored using the Fluidigm SNP Genotyping Analysis Software version 3.1.1.

Standardized parental genotypes were stored on a Progeny database server housed at EFGL. Progeny software (http://www.progenygenetics.com/) is already used by the majority of GAPS labs throughout the Pacific Northwest: Idaho Department of Fish and Game, University of Washington, NOAA-Northwest Fisheries Science Center, Washington Department of Fish and Wildlife, Columbia River Intertribal Fish Commission, and U.S. Fish and Wildlife Service.

Data quality was inferred from estimates of completion rate, missing data, poor performing loci, and error rates. The program ML-NULLFREQ (Kalinowski and Taper 2006) was used to identify loci with null alleles and estimate the proportion of null alleles per locus. Basic diversity indices were calculated for the brood years. This included estimates of average heterozygosity (observed Ho and expected He) using ARLEQUIN (Excoffier and Lischer 2010), genetic structure (Fst and assignment tests) using GENEPOP (Rousset 2008) and ONCOR (Kalinowski et al. 2007), and effective population size (Ne) using LDNE (Waples and Do 2008).

Sex locus

In an effort to increase the accuracy of the Chinook sex-determining SNP assay, a modified assay was used beginning with SY2010. For each hatchery stock that was genotyped with the modified marker, comparisons were made between the phenotypic sex of samples, which was determined at time of spawning, and the genetically determined sex of samples. The accuracy of a newly modified steelhead sex-determining SNP assay was likewise evaluated for stocks in SY2010 that were genotyped with the new assay.

Tagging rate

Because genotypes from 100% of the broodstock are not always obtained for all hatchery stocks, this results in a small portion of hatchery-origin offspring that are genetically "un-tagged." This "un-tagged" portion of hatchery-origin fish cannot be assigned back to their parental pair or hatchery of origin because genotypes are missing from one or both of their parents and genotypes from both parents are needed for accurate PBT assignment. However, we can easily estimate the proportion of "untagged" progeny of each hatchery stock for each brood year based on the proportion of successfully genotyped broodstock. The proportion of "tagged" progeny is not equal to the proportion of successfully genotyped broodstock, but rather the product of the proportion of successfully genotyped males and the proportion of successfully genotyped females. Thus, if *f* is the proportion of female spawners that are genotyped and *m* is the proportion of male spawners genotyped, then the proportion of PBT-tagged offspring is expected to be *mf.* Additionally, assuming that males and females are successfully genotyped at equal rates, the proportion of PBT-tagged offspring can also be estimated by squaring the total proportion of successfully genotyped broodstock. In this report, we use this latter method to estimate the proportion of PBT-tagged offspring from each stock (Tables 3 and 4). In the future, for estimates of tagging rate, we will determine the proportion of tagged progeny by simply enumerating the proportion of crosses for which both parents have been successfully genotyped. This direct approach is expected to provide a more accurate calculation of tagging rate rather than estimating the tagging rate based on proportions of successfully genotyped broodstock. For cases in which cross information is not recorded, we will determine the tagging rate of a stock using the previously described method of using the proportion of successfully genotyped males and females.

Results

Completion rate and missing data

If a sample failed to genotype at 10 or more SNPs it was re-extracted and regenotyped. If that sample failed a second time at 10 or more SNPs, it was automatically excluded from future PBT analyses because the excess missing data prevents accurate parentage assignment. However, samples with missing data for 10 or more SNPs were not excluded from the summary statistics presented in this report, because useful information about population structure and performance of the SNP assays can still be gleaned from such samples.

For steelhead SY2010, all 5,282 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 5,282 samples, 5,198 (98.4%) were genotyped with an acceptable level of missing data (Table 3). In this final SY2010 PBT baseline comprising the remaining 5,198 samples, there were just 2,168 missing genotypes due to SNP failure out of a possible 493,810 genotypes. This resulted in missing data for just 0.44% of the genotypes.

For steelhead SY2011, all 5,533 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 5,533 samples, 5,379 (97.2%) were genotyped with an acceptable level of missing data (Table 3). In this final SY2011 PBT baseline comprising the remaining 5,379 samples, there were 15,526 missing genotypes due to SNP failure out of a possible 511,005 genotypes. This resulted in missing data for just 3.0% of the genotypes.

For Chinook SY2008, all 9,782 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 9,782 samples, 9,686 (99.0%) were genotyped with an acceptable level of missing data (Table 4). In this final SY2008 PBT baseline comprising the remaining 9,686 samples, there were just 5,120 missing genotypes due to SNP failure out of a possible 920,170 genotypes. This resulted in missing data for just 0.55% of the genotypes.

For Chinook SY2009, all 8,776 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 8,776 samples, 8,349 (95.14%) were genotyped with an acceptable level of missing data (Table 4). In this final SY2011 PBT baseline comprising the remaining 8,349 samples, there were 11,616 missing genotypes due to SNP failure out of a possible 793,155 genotypes. This resulted in missing data for just 1.47% of the genotypes.

For Chinook SY2010, all 8,290 samples were extracted and genotyped with 95 PBT SNPs and the sex-identification assay. Of the 8,290 samples, 8,235 (99.3%) were genotyped with an acceptable level of missing data (Table 4). In this final SY2010 PBT baseline comprising the remaining 8,235 samples, there were just 6,439 missing genotypes due to SNP failure out of a possible 782,325 genotypes. This resulted in missing data for just 0.82% of the genotypes.

Poor performing loci

Of the samples that genotyped with <10 missing SNPs, poor performing SNP assays were identified within the 95 PBT SNP panel.

For steelhead SY2010, two loci failed to genotype at >3% of samples. Locus Omy_Il1b-198 failed at 213 (4.1%) of the samples, OMS00039 failed to genotype 164 (3.2%) of the samples.

For steelhead SY2011, the same two loci failed to genotype >3% of samples. Locus Omy_Il1b-198 failed at 266 (5.0%) of the samples, OMS00039 failed to genotype 218 (4.1%) of the samples. All other loci failed at <3% of samples.

For Chinook SY2008, only a single locus failed at >3% of samples. Locus Ots_pigh-105, failed to genotype at 2,915 (30.1%) of the samples.

For Chinook SY2009, there were nine loci that failed at >3% of the samples. Ots_pigh-105 failed at 2218 (26.6%) of samples, Ots_94903-99R failed at 775 (9.3%) of samples, Ots_CD59-2 failed at 742 (8.9%), Ots_96500-180 failed at 744 (8.9%) of samples, Ots_ARNT failed at 746 (8.9%) of samples, Ots_AsnRS-60 failed at 744 (8.9%), Ots_brp16-64 failed to genotype 727 (8.7%) of samples, Ots_CirpA failed at 730 (8.7%) of samples, and Ots_96899-357R failed at 727 (8.7%) of samples.

For Chinook SY2010, two loci failed at >3% of the samples. Locus Ots_Ikaros-25 failed at 758 (9.1%) of samples and Ots_txnip-321 failed at 548 (6.6%).

Error rate (quality control)

For steelhead SY2010, a subset of 222 samples representing all extraction plates were regenotyped and checked for discrepancies with original PBT genotypes in order to estimate genotyping error rates. This resulted in 21,090 rerun genotypes being compared to the original genotypes. Of these genotypes, 139 had a SNP failure either in the original genotype or the rerun genotype and could not be used in error estimation. This resulted in 20,951 genotypes with three discrepancies between the original and samples and a genotyping error rate of 0.014%.

For steelhead SY2011, a subset of 207 samples representing all extraction plates were rerun and checked for discrepancies. This resulted in 19,665 rerun genotypes being compared to the original PBT genotypes. Of these genotypes, 398 had a SNP failure either in the original genotype or the rerun genotype and could not be used in error estimation. This resulted in 19,267 genotypes with 93 discrepancies between the original and samples and a genotyping error rate of 0.48%.

For Chinook SY2008, a subset of 431 samples representing all extraction plates were rerun and checked for discrepancies. This resulted in 40,945 rerun genotypes being compared to the original PBT genotypes. Of these genotypes, 756 had a SNP failure either in the original genotype or the rerun genotype and could not be used in error estimation. This resulted in 40,189 genotypes with 59 discrepancies between the original and samples and a genotyping error rate of 0.15%.

For Chinook SY2009, a subset of 386 samples representing all extraction plates were rerun and checked for discrepancies. This resulted in 36,670 rerun genotypes being compared to the original PBT genotypes. Of these genotypes, 1,749 had a SNP failure either in the original genotype or the rerun genotype and could not be used in error estimation. This resulted in 34,921 genotypes with 185 discrepancies between the original and samples and a genotyping error rate of 0.53%.

For 2010 Chinook, a subset of 348 samples representing all extraction plates were rerun and checked for discrepancies. This resulted in 33,060 rerun genotypes being compared to the original PBT genotypes. Of these genotypes, 754 had a SNP failure either in the original

genotype or the rerun genotype and could not be used in error estimation. This resulted in 32,306 genotypes with 175 discrepancies between the original and samples and a genotyping error rate of 0.54%.

Null alleles

For steelhead SY2010, 53 of the 95 PBT loci were found to have a frequency of null alleles greater than zero, but only five loci had frequencies >5% (Table 5). For steelhead SY2011, 46 of the 95 PBT loci were found to have a frequency of null alleles greater than zero, but only four loci had frequencies greater than 5% (Table 6). For Chinook SY2008, 58 of the PBT loci were found to have a frequency of null alleles greater than zero, but only one locus had a frequency >5% (Table 7). For Chinook BY2009, 72 of the PBT loci were found to have a frequency of null alleles greater than zero, but only 1 locus had a frequency >5% (Table 8). For Chinook BY2010, 48 loci were identified as having null alleles, two of which occurred at frequencies >5% (Table 9).

Sex markers

The sex-specific assay for steelhead matched phenotypic sex in 94.4% of the samples (Table 10). For instances in which genetically-determined sex did not correspond to the phenotypic sex, all were cases in which phenotypic females were misidentified by genotype as males. The assay either failed to genotype or provided ambiguous results for 1.8% of the samples.

The sex-specific assay for Chinook salmon matched phenotypic sex in 100% of the samples (Table 11). The assay produced ambiguous results, or failed to genotype, 3.2% of samples.

Average He

Levels of observed heterozygosity within steelhead broodstocks was ~0.4 for all hatcheries and were similar among hatchery broodstocks and across years (Table 12). Levels of observed heterozygosity tended to be lower in Chinook (~0.35) in all stocks and across brood years (Table 13).

Population structure (Fst)

Pairwise Fst was calculated among the steelhead SY2010 and SY2011 hatchery stocks (Tables 14 and 15). Values ranged from a low of 0.005 between Touchet and Tucannon in SY2010 and a low of <0.001 between Touchet and Tucannon in SY2011 to a high of 0.072 between Dworshak and Little Sheep Crk. in SY2010 and a high of 0.071 between Squaw Crk and Little Sheep Crk. in SY2011. All Fst values among stocks were significant within each year. For Chinook SY2008 pairwise Fst values ranged from a low of 0.001 between the NPFH samples and Dworshak to a high of 0.057 between Pahsimeroi and Tucannon (Table 16). Chinook SY2009 had Fst levels ranging from a low of 0.0004 between the NPFH stock and the Dworshak stock to a high of 0.63 between the Grande Ronde stock and the Tucannon stock (Table 17). Pairwise Fst values for Chinook BY2010 ranged from a low of 0.003 between Powell and NPFH and a high of 0.055 between Tucannon and Pahsimeroi (Table 18).

Inferences about population structure were also examined by conducting a leave-one-out test conducted in ONCOR (Kalinowski et al. 2007). This test determines the proportion of

fish in a population that can be assigned back to their population of origin and identifies the most common population that fish are misassigned to. A high proportion of individuals from both the steelhead SY2010 and SY2011 broodstocks assigned back to their population of origin (Tables 19 and 20). Populations that fish most often misassigned to were stocks with shared population histories. Similar patterns were observed for Chinook SY2008, SY2009, and SY2010 (Tables 21–23).

Effective population size (Ne)

Effective population size (Ne) and 95% CI for each steelhead hatchery stock in SY2010 and SY2011 was calculated (Table 24). Estimates of effective population size ranged from a low of 31.9 for East Fk. Salmon R. in SY2010 and a low of 35.3 for Squaw Crk. in SY2011 to a high of 313.2 for Dworshak in SY2010 and a high of 254.3 for Little Sheep Crk in SY2011. In SY2011 Touchet had an estimate of 702.5 an infinitely large CI which suggests that the estimate is inaccurate.

Effective population size and 95% CI for each Chinook hatchery stock in SY2008, SY2009, and SY2010 was also calculated (Table 25). Estimates of effective population size ranged from a low of 38.9 for Catherine Crk in SY2008 to a high of 753.5 for Rapid River in SY2009. Johnson Crk had an estimated Ne of 2,931.8, but had an infinitely large CI, which suggests that the estimate is inaccurate.

Discussion

We have demonstrated the ability to routinely genotype the thousands of broodstock samples collected each year. Genotypes are stored and organized in an on-site database where they can be exported for PBT analysis. The creation of these PBT baselines also provides several measures of genetic diversity and relatedness among the broodstocks, which provide the added benefit of genetic monitoring of hatchery populations. The completion of this objective allows parental genotypes to be queried in parentage analyses resulting in the identification of hatchery fish originating from the Snake River basin.

Completion rate and missing data

The high rate of genotyping success for samples and the low rate of missing data demonstrates the feasibility of collecting high quality data from nearly all Snake River basin broodstock samples.

Poor loci

Previously, the Omy_Ogo4-212 locus had performed poorly in the steelhead SY2008 and SY2009 broodstocks (Steele et al. 2011). A redesigned version of the locus has performed well and provides consistent quality genotypes since being included in the 2010 broodstock.

One of the two poor-performing loci (OMS00039) within steelhead SY2010 and SY2011 is known to have null alleles. To prevent null allele genotypes from being included in the database we have adopted scoring rules for these loci. If clustering patterns of samples at these loci suggest the presence of null alleles then the genotypes are manually ‘no called’, meaning that the genotype is not scored nor included in the data in order to minimize including null allele genotypes. The high proportion of failed samples at this locus is likely due to conservative scoring of genotypes.

Within the Chinook SY2008 just a single locus, Ots_pigh-105, had a high rate of failure (30.1%). This locus also had a high failure rate (26.6%) in SY2009. Because of the poor rate of genotyping success with this locus, it was redesigned. Subsequently, the assay has performed well and successfully genotyped >98% of samples within Chinook SY2010. The high failure rates of the remaining eight loci within Chinook SY2009 were determined to have been caused by the manual removal of their genotypes from a large subset of the data. It was determined that lab error had compromised the accuracy of these assays for some samples, thus the genotypes were removed from the dataset resulting in a perceived increase in the failure rate for these assays. Two loci within Chinook SY2010 had high levels of failure. For one locus, Ots_Ikaros-25, the pre-amp primers were inadvertently excluded from the assay and likely contributed to poor amplification resulting in a large number of failed samples. The second locus, Ots_txnip-321, has poor clustering patterns after we transitioned to fast thermocyclers and many samples were no called because of ambiguous results.

Error rate (quality control*)*

To minimize false negatives in parentage assignments, genetic markers need to exhibit low genotyping error rates and researchers should accommodate estimated error rates during data analysis (Kalinowski et al. 2007). Genotyping error rates for microsatellite markers are variable, but have often been reported between 1-2% (Pearse et al. 2009; Hauser et al. 2011). For the parentage software programs CERVUS and SNPPIT, the default error rate used is 1%. We consistently observed error rates ≤1% for both the steelhead and Chinook PBT panels of SNPs across several years.

Null alleles

Three of the five steelhead PBT loci that had frequencies of null alleles >5% (OMS00118, Omy_vatf406, Omy_113490159) are the same loci that had similar levels of null alleles in SY2008 and SY2009. These loci may need to be reevaluated or scoring rules for the loci may need to be modified to account for null alleles.

Within the Chinook SNP panel the locus Ots_OTALDBINT1SNP1 was identified as having a null allele frequency >5% for SY2008, SY2009, and SY2010. This locus may need to be re-evaluated because of consistent presence of null alleles. In SY2010, the locus Ots_Ikaros250 had a very high frequency of null alleles (12%). This is almost certainly due to the overall poor performance of this SNP within SY2010, which was likely caused by the inadvertent exclusion of pre-amp primers for this assay during the initial amplification of the locus. Considering that this locus was not identified as having null alleles in neither SY2008 nor in SY2009 the high estimate of null alleles in SY2010 is likely due to poor amplification within this brood year and not an inherent problem with the SNP assay.

Sex markers

Sex-specific assays for both species performed very well. The modified steelhead sex assay had a high level of genotyping completeness and failed to produce genotypes for just 1.8% of samples. The Chinook salmon sex assay had a slightly higher failure rate of 3.8% but this is a marked improvement over the 17.9% failure rate of the previous assay (Steele et al. 2011). Overall, the results are generally encouraging in that these modified assays can provide an accurate and nonlethal method of sex determination for these species.

Average He

The average expected heterozygosity was high and uniform across both steelhead hatchery stocks (~40%) and Chinook (~35%) demonstrating that the degree of variability in these SNP sets makes them useful for parentage analysis of hatchery stocks throughout the Snake River basin.

Population structure

Within steelhead, the highest pairwise Fst values are consistently seen between the Dworshak Hatchery stock and other locations, except with the Squaw Creek source, which is derived from Dworshak stock. The larger degree of divergence between Dworshak and the other stocks reflects the distinctness of Clearwater origin fish to those in the Salmon and Snake rivers. The lowest Fst values are also consistently seen between populations that are geographically proximate, such as the Touchet and Tucannon stocks in Washington State, or among stocks with shared populations histories, such as the Oxbow, Sawtooth, and Pahsimeroi stocks (Tables 14 and 15). Low divergence among Oxbow, Sawtooth, and Pahsimeroi reflect their shared history of being recently derived from stocks whose brood source came from wild adult steelhead trapped at the Hells Canyon Dam on the Snake River in the late 1960s (Nielsen et al. 2009). Within Chinook, the lowest Fst values tended to be among stocks within the Clearwater drainage (Dworshak, Powell, Nez Perce, and Clearwater) while the highest tended to be among the most geographically distant stocks (Sawtooth/Pahsimeroi and Tucannon).

Patterns from the leave-one-out-tests conducted in ONCOR also reflect the shared population history of the hatchery stocks. The large amount of misassignment in steelhead BY2010 between the Grande Ronde and Wallowa stocks or the Touchet and Tucannon stocks reiterates the close relatedness among these pairs of stocks (Table 19-20). Similarly, high rates of misassignment were seen between Chinook stocks with common population histories or stocks that are geographically proximate to one another. Examples include a 20.4% misassignment rate of the Clearwater stock to the Powell stock and an 18.2% misassignment rate of Johnson Crk. stock to the South Frk. Salmon within SY2008, and a 15.6% misassignment rate of the Catherine Crk stock to the Lookingglass stock or the 12.3% misassignment rate of the Powell stock to the Dworshak stock in BY2010 (Table 21). These misassignments of hatchery stocks emphasize the need for a PBT approach to determine the origin of hatchery stocks, as GSI approaches would be unable to distinguish among them.

Effective population size (Ne)

Effective population sizes generally corresponded to size of broodstock. Larger hatchery programs (e.g., steelhead stocks at Dworshak, Oxbow, Pahsimeroi, Sawtooth, and Wallowa or Chinook stocks of Clearwater and Rapid River) tended to have larger Ne, while programs with smaller broodstocks (steelhead stocks of Squaw Crk and EFSR or Chinook stocks of Lookingglass and Catherine Crk.) had a smaller Ne. Estimates of Ne for Johnson Crk in SY2008 yielded an infinitely large CI interval which, while making the estimate of Ne imprecise, indicates no evidence of linkage disequilibrium for this population.

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SECTION 3: UTILIZATION OF PBT METHODS TO PROVIDE PARENTAL ASSIGNMENTS

Introduction

Several years' worth of broodstock genotypes have now been collected for both steelhead and spring/summer Chinook. Projects can now assess the accuracy and utility of PBT in addressing a multitude of conservation and management questions involving hatchery stocks. We report the results from three projects that have utilized the PBT database: 1) Comparisons of stock and cohort assignments between CWT and PBT, 2) Identifying the origin of hatchery strays within the Deschutes River, and 3) Identifying the origin of out-migrating hatchery kelt steelhead in the Snake River. Additional projects are underway that will utilize the PBT database and include 1) Composition of Snake River hatchery fish harvested in the Zone 6 Columbia River fishery, and 2) Run reconstruction of hatchery fish returning over Lower Granite Dam. Results on these ongoing projects will be presented in the next annual report.

Methods

Samples collected for various "back end" projects were inventoried and genotyped using the same procedures as the broodstock. The program SNPPIT was used to conduct parentage analysis. Unless indicated otherwise, the criteria for accepting a PBT assignment was an FDR (False Discovery Rate) of <1%.

Comparison between CWT and PBT assignments in steelhead

As part of hatchery evaluation efforts by the Lower Snake River Compensation Plan (LSRCP) snouts of steelhead with CWTs were collected in the 2010 Idaho fishery. Snouts were sent to the IDFG coded-wire lab in Nampa for processing. A tissue sample was taken from the snout for PBT analysis as the CWTs were being excised. The CWT-determined origin of the steelhead was then compared to the PBT-determined origin.

Comparison between CWT and PBT assignments in Chinook

A total of 186 tissue samples were collected from adult hatchery Chinook salmon with coded wire tags (CWTs) during a creel survey conducted in the Salmon (n = 85) and Clearwater River (n = 101) basins by Idaho Department of Fish and Game in 2011 and genotyped with the PBT SNP panel. Hatchery of origin and age data were determined by CWTs. Based on CWT data, approximately 45% (83 of the 186) of individuals were expected to assign to hatchery parents using PBT. Specifically, age 3 individuals originating from a Snake River hatchery (i.e., spawn year 2008) should assign to parents in the 2008 parental baseline. Older age classes (age 4 or 5) should not assign to parents because their parents predate the collection of PBT baselines in the Snake River basin.

Origin of hatchery stray steelhead in the Deschutes River

In July through October 2011, 1,779 returning adult wild and hatchery steelhead were captured in the Sherars Falls trap, located at river mile 43 in the Deschutes River, Oregon. Wild or hatchery status was determined by Oregon Department of Fish and Wildlife biologists based on adipose fin presence or absence and any other identifying marks. Tissue samples were collected from 750 hatchery- and wild-origin individuals for genetic analysis. Trios (offspring assignment to two parents) were accepted based on a combination of criteria including: number of Mendelian incompatibilities, hatchery cross records, false discovery rate (FDR), and *p*-value

associated with the parentage assignment. Any trio with an associated FDR greater than 1% and *p*-value greater than 0.05 were only accepted parentage if there were no Mendelian incompatibilities, and/or the parent pair was supported by hatchery cross records.

Origin of steelhead kelts sampled at Lower Granite Dam

Genetic samples from 528 ad-clipped hatchery kelts were collected in 2011 as they out-migrated over Lower Granite Dam. Kelts were trapped at the dam's juvenile bypass facility and were genetically sampled before being released downstream. Parentage assignment was conducted using both the BY2008 and BY2009 broodstock as potential parents.

Results

CWT/PBT comparison in steelhead

Because the genotyping of parental broodstock began in 2008 only 1-ocean steelhead sampled for this project are expected to assign to the PBT baseline. Results from CWTs indicated that 61 of the sampled snouts originated from the 2008 broodstock. Of those 61 samples, 59 were successfully genotyped. Of the 59 samples, 52 (88.1%) assigned to a broodstock parents (Table 26), all PBT assignments matched the CWT-determined origin and thus concordance was 100% between the two methods. The seven samples that did not assign to any parents comprised five samples from Oxbow and 2 from Dworshak.

CWT/PBT comparison in Chinook

Of the 83 individuals that were expected to assign to parents, we confidently assigned 68, or 82% (Table 27). One individual (out of the 103) that was not expected to assign to parents based on age given by CWT (4-year-old), confidently assigned to parents that were spawned at the Powell facility in the Clearwater River in 2008. The 69 assignments met a threshold criteria of FDR <2.5%, p <0.05, and were incompatible at no more than one locus within the trio (offspring assignment to both parents). Although three individuals did not meet the assignment criteria above, we accepted their parentage assignment based on a combination of zero Mendelian incompatibilities with one parent, and confirmation with hatchery spawn records indicating that one parent was not included in the baseline due to missing data. In addition, hatchery of origin given by CWT and PBT results were concordant for these three individuals, thereby bringing the percent of fish assigned with PBT to 86%. The CWT and PBT approaches revealed 100% concordance in identifying the Snake River hatchery of origin for each of the 72 fish.

Origin of hatchery stray steelhead in the Deschutes River

Of the 750 samples, a total of 724 individuals (446 hatchery, 278 wild origin) were successfully genotyped. Parentage assignment using the SY2008 steelhead broodstock baseline was then conducted. Only age three hatchery origin fish originating from sampled Snake River hatcheries should assign to parents. Of the 724 individuals in the dataset, assignments were made for 124 (or 29% of hatchery origin fish) to two hatchery broodstock parents spawned in 2008 at Snake River hatcheries (Table 28). Age three hatchery strays to the Deschutes River largely originated from four Snake River hatcheries: Dworshak (n = 28), Oxbow (n = 34), Pahsimeroi (n = 33), and Sawtooth (n = 30). Two fish, one originating from Pahsimeroi and the other from Sawtooth hatchery, were unmarked (not adipose clipped) hatchery origin strays.

Origin of steelhead kelts sampled at Lower Granite Dam

Of the 528 samples, 71 failed to be genotyped with sufficient data resulting in 457 samples (86.6%) being analyzed. Of those remaining samples, 166 (36.3%) assigned to two parents from the SY2008 PBT database. Only age three hatchery origin fish originating from sampled Snake River hatcheries should assign to parents. The majority of the assigned kelts originated from the Sawtooth (n = 71) and Pahsimeroi (n = 61) hatcheries (Table 29). All samples assigned back to hatchery parents spawned in the 2008 brood year except one individual that assigned to the Sawtooth broodstock from the 2009 brood year.

Discussion

CWT/PBT comparison in steelhead

PBT was successful in assigning 88.1% of fish to parents, with 100% concordance between CWT and PBT hatchery of origin. Additionally, another indication of success is that there were no misassignments using PBT. In other words, fish that did not have parents in the dataset, such as fish originating from the 2007 broodstock or unsampled broodstocks, did not falsely assign to any parental pair in the dataset from another hatchery.

The non-assignment of the two Dworshak samples is likely due to unsampled parents. Early egg takes at Dworshak were missed in 2008. These egg takes represent 240 fish, or 14.5% of the potential parents, from that stock. This means that 83.1% of the Dworshak broodstock was sampled, which translates to a 73.2% tagging rate for that stock. We observed a similar assignment rate (77.8%) of Dworshak-origin fish, suggesting that the non-sampled broodstock likely included the parents of the two non-assigned Dworshak samples.

The reason for non-assignment of the four Oxbow samples is unclear. The Oxbow stock had the largest number of failed samples for the 2008 PBT dataset. There were 32 (3.45%) samples that failed to genotype, which translates to a tagging rate of 93.2% for this stock. However, this does not completely explain the lower assignment of Oxbow-origin samples. However, 26 of the 32 failed Oxbow samples were female and during brood year 2008 it was standard practice at Oxbow to split the eggs of a female and fertilize each half with a different male. If there are 26 missing females this would represent 52 crosses from which the resulting progeny would not assign because the mother is missing from the database, thereby potentially lowering the tagging rate for this stock. While the reason for non-assignment for a proportion of the Oxbow samples cannot be adequately explained, it appears to be a stock-specific anomaly. The lower assignment rate for Oxbow-origin samples may also simply represent sampling error in which assignment rates would increase as more Oxbow-origin samples are run.

Overall, PBT performed well and correctly assigned samples when assignments could be made. Additionally, PBT did not misassign any of the samples that did not originate from the 2008 broodstock. Both of these results provide a good indication that PBT would perform well when using samples of unknown origin

CWT/PBT comparison in Chinook

Seventy-two individuals (86% of expected assignments) sampled in the 2011 creel survey were assigned to parents in the 2008 PBT parental baseline. Concordance was 100%

between the CWT and PBT approaches as each method identified the same Snake River hatchery of origin for each of these 72 fish. Because one individual assigned with confidence to parents at the Powell facility in the Clearwater River, we conclude that the fish was misidentified as a 4-year-old via CWT analysis. Misidentification in age may be one reason why 12 fish did not assign to parents in the baseline despite the expectation of assignment based on age ("3-year-old" via CWT). Alternatively, the parent(s) for the 12 unassigned individuals may not be in the parental baseline due to incomplete genetic data, or a tissue sample was not obtained. This later scenario is almost certainly responsible for not assigning any of the CWT samples originating from the Lookingglass Hatchery. Broodstock for the Grande Ronde, Lostine, and Catherine Crk stocks that are collected as returning adults are spawned at Lookingglass Hatchery and are sampled at 100%. However, captive broodstocks also exist for these populations and they are spawned at off-site locations (Bonneville Hatchery in Oregon and the Manchester Research Station in Washington State) before being transferred to Lookingglass Hatchery for marking and release. These captive broodstocks, which are unsampled, may contribute a substantial portion of the smolts released for each of the three populations. Efforts are now underway to include samples from these captive broodstocks in the PBT database. Captive broodstocks for the Lostine and Catherine Creek are being phased out but will continue for the Grande Ronde stock (USFWS 2011). Nevertheless, the PBT approach proves to be powerful at accurately identifying the correct hatchery of origin and age of sampled individuals as validated by CWTs.

Origin of hatchery stray steelhead in the Deschutes River

Approximately 29% of the sampled steelhead of hatchery origin returns to the Deschutes River in 2011 assigned to parents that were spawned as broodstock at Snake River hatcheries in 2008. Parentage assignment is only possible for the offspring from broodstock that were included in the parental database. Therefore, several reasons exist for why the remaining 71% of hatchery origin fish did not assign to parents in our database: 1) Strays may originate from hatcheries outside the Snake River basin (i.e., parents were not in the database); 2) Incomplete genetic data for at least one parent, or incomplete sampling of broodstock parents; and 3) Individual offspring may be a stray originating from the Snake River but represents a different age class (i.e., age 4 or 5). The latter is likely to represent the main reason for unassigned hatchery strays, because the parental database was initiated in 2008 and could only identify offspring at age 3 at the time of genetic analysis. As the parental database expands each year, the ability to assign Snake River origin hatchery strays in the Deschutes River will improve in future years.

Origin of steelhead kelts sampled at Lower Granite Dam

While 36% of the hatchery kelts assigned to parents with PBT, we expect a larger proportion of samples to assign in subsequent years when more age classes are represented in the PBT baseline. Only 1-ocean fish assigned, because 2-ocean fish or older would originate from broodstocks that predated PBT sampling. Several broodstocks were also unsampled in 2008 including the large Wallowa stock in Oregon (~450 spawners) and the Lyons Ferry stock in Washington (~300 spawners). Other smaller stocks, including the Touchet and Tucannon stock in Washington, were also missed in 2008. In addition, a portion of the Dworshak stock (14.5%) was also unsampled in BY2008. All of these missing stocks were sampled completely at 100% beginning in BY2009 and should result in higher PBT assignment rates.

The single fish that assigned to the Sawtooth SY2009 broodstock is likely to not be a kelt from the SY2011 but rather a very early returning individual from the subsequent run of SY2012

fish. The parents identified using PBT for this sample matched hatchery cross records indicating that the assignment is correct. This fish was one of the smallest fish encountered during sampling (FL = 540 mm) and was sampled on one of the last collection days at the end of June. The fish likely fell back over the dam and was trapped along with the out-migrating kelts from SY2011.

Organizational changes are being made at rearing facilities that will soon allow PBT-tagged fish to be tracked to their release site. In the future this program will not only identify the hatchery of origin for hatchery kelts but also their release location. This will allow the role of off-site releases in hatchery kelts to be investigated.

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ACKNOWLEDGEMENTS

The scope and magnitude of this project could not have been completed without the help and cooperation of many individuals from many different agencies and organizations. Thanks to Brian Leth, Sam Sharr, and Carl Stiefel from IDFG; Jeff Stephenson, Amanda Matala, Vanessa Morman, Stephanie Harmon, Megan Moore, Travis Jacobsen, Nick Hoffman, and Lori Maxwell from the Columbia River Inter-Tribal Fish Commission; and Carlos Camacho, Dylan Kovis, Laura Redfield, and Thea Vanderwey from the Pacific States Marine Fisheries Commission (IDFG Eagle Fish Genetics Lab). Special thanks to all IDFG, ODFW, WDFW, USFWS, NPT staff and volunteers for help taking broodstock samples at hatcheries. Cheryl Zink's help with report formatting and editing is also appreciated. We also appreciate Barbara Shields' help in managing contract and reporting requirements. Primary funding for this project comes from the Bonneville Power Administration (#2010-031-00). Additional financial support was provided by the Pacific Coast Salmon Recovery Fund and the Idaho Power Company (Project #01608SC).

TABLES

Table 1. Total steelhead hatchery broodstock genetically sampled in spawn years 2010 and 2011 in the Snake River basin. Broodstock are sampled at 100% but only samples from broodstock producing offspring were included (i.e. samples from broodstock whose eggs were culled were not included).

Snake River Hatchery	SY2010	SY2011
LSRCP/IDFG Sawtooth (IDFG & SBT)	860	824
LSRCP/IDFG Sawtooth (EFSR)	136	100
LSRCP/IDFG Sawtooth (USB/Squaw)	45	49
Idaho Power/IDFG, Oxbow F.H.	524	396
Idaho Power/IDFG, Pahsimeroi F.H.	814	814
Idaho Power/IDFG, Pahsimeroi F.H (SBT)	288	266
LSRCP/IDFG/USFWS Dworshak/C.W.	1644	2018
LSRCP/ODFW-Wallowa F.H.	500	484
LSRCP/WDFW-Lyons Ferry	198	176
LSRCP/ODFW- Little Sheep Crk	107	127
LSRCP/WDFW-L.F. (Tucannon)	34	38
LSRCP/WDFW-L.F. (Touchet)	28	25
LSRCP/WDFW-L.F. (G.R. cottonwood)	104	191
Total	5282	5508

Table 2. Total Chinook salmon hatchery broodstock sampled in spawn years 2010 and 2011 in the Snake River basin. Broodstock are sampled at 100% but only samples from broodstock producing offspring were included (i.e. samples from broodstock whose eggs were culled were not included).* Information not available at time of reporting.

Snake River Hatchery	SY2010	SY2011
Idaho Power/IDFG, Rapid River	2344	2012
LSRCP/USFWS, Dworshak	1237	1399
LSRCP/IDFG, Clearwater (Powell)	424	962
LSRCP/IDFG, Clearwater (SF)	762	676
LSRCP/IDFG, Sawtooth	677	324
Idaho Power/IDFG, Pahsimeroi River	555	707
LSRCP/WDFW-L.F. (Tucannon)	162	167
LSRCP/IDFG, McCall (SFSR)	880	909
LSRCP/ODFW, Imnaha	245	255
LSRCP/ODFW/NPT, Lostine	129	123
LSRCP/ODFW, Catherine Crk.	74	75
LSRCP/ODFW, Grande Ronde	155	74
LSRCP/ODFW, Lookingglass Crk.	155	148
Nez Perce Tribal Hatchery (NPTH)	491	356
Johnson Crk. (EFSR Salmon River)	70	85
Total	8360	8252

Table 3. Sample sizes and genotyping completion rate of SY2010 and SY2011 steelhead broodstock. Samples with ≥10 failed PBT SNPs are not consider to be successfully genotyped. The PBT-tagging rate for each stock is calculated by squaring the proportion of successfully genotyped broodstock.

	2010			2011		
Snake River Hatcheries	**Sampled**	**Genotyped (%)**	**Tagging Rate**	**Sampled**	**Genotyped**	**Tagging Rate**
LSRCP/IDFG - Sawtooth (IDFG & SBT)	860	859 (99.9%)	99.77%	824	823 (99.9%)	99.77%
LSRCP/IDFG - Sawtooth (EFSR)	136	136 (100%)	100.00%	100	100 (100%)	100%
LSRCP/IDFG - Sawtooth (USB/Squaw)	45	44 (97.8%)	95.60%	49	49 (100%)	100%
Idaho Power/IDFG - Oxbow Hatchery	524	512 (97.7%)	95.47%	396	379 (95.7%)	91.60%
Idaho Power/IDFG - Pahsimeroi Hatchery	814	802 (98.5%)	97.07%	814	728 (89.4%)	79.99%
Idaho Power/IDFG - Pahsimeroi Hatchery (SBT)	288	275 (95.5%)	91.18%	266	241 (90.6%)	82.09%
LSRCP/IDFG/USFWS - Dworshak/Clearwater	1644	1620 (98.5%)	97.10%	2018	2010 (99.5%)	98.91%
LSRCP/ODFW - Wallowa	500	494 (98.8%)	97.61%	484	480 (99.2%)	98.35%
LSRCP/ODFW - Wallowa (Little Sheep)	107	101 (94.4%)	89.10%	127	127 (100%)	100%
LSRCP/WDFW - Lyons Ferry	198	198 (100%)	100.00%	176	175 (99.4%)	98.88%
LSRCP/WDFW - L.F. (Tucannon)	34	30 (88.2%)	77.85%	38	32 (84.2%)	70.91%
LSRCP/WDFW - L.F. (Touchet)	28	23 (82.1%)	67.47%	25	34 (100%)	100%
LSRCP/WDFW - L.F. (G.R. cottonwood)	104	104 (100%)	100.00%	191	187 (97.9%)	95.86%
Total	5282	5198 (98.4%)	96.83%	5508	5365 (97.4%)	94.88%

Table 4. Sample sizes and genotyping completion rate of SY2008-SY2010 Chinook broodstock. Samples with ≥10 failed PBT SNPs are not considered to be successfully genotyped. The PBT-tagging rate for each stock is calculated by squaring the proportion of successfully genotyped broodstock. (†) Indicates information not available at time of reporting, (*) indicates stocks in some years were not sample at 100%. In these cases, the fraction of sampled broodstock is reported, the proportion genotyped reflects the genotyping success rate of the sampled broodstock, and the tagging rate reflects the overall rate for the stock.

	2008			2009			2010		
Snake River Hatcheries	**Sampled**	**Genotyped (%)**	**Tagging Rate**	**Sampled**	**Genotyped (%)**	**Tagging Rate**	**Sampled**	**Genotyped (%)**	**Tagging Rate**
Idaho Power/IDFG - Rapid River	2866	2843 (99.2%)	98.40%	2099	2041(97.24%)	94.55%	2344	2333 (99.5%)	99.06%
LSRCP/USFWS - Dworshak	1213	1198 (98.8%)	97.54%	1180	1176(99.7%)	99.32%	1237	1227 (99.2%)	98.39%
LSRCP/IDFG - Clearwater (Powell)	902	896 (99.3%)	98.67%	851	592(69.57%)	48.39%	424	419 (98.8%)	97.66%
LSRCP/IDFG - Clearwater (SF)	1029	1013 (98.5%)	96.91%	846	789 (93.26%)	86.98%	762	760 (99.7%)	99.48%
LSRCP/IDFG - Sawtooth	1186	1180 (99.5%)	98.99%	979	977(99.8%)	99.59%	677	675 (99.7%)	99.41%
Idaho Power/IDFG - Pahsimeroi	714	708 (99.2%)	98.33%	628	621(98.89%)	97.78%	555	551 (99.3%)	98.56%
LSRCP/WDFW - L.F. (Tucannon)	114	108 (94.7%)	89.75%	175	169(96.57%)	93.26%	162	159 (98.2%)	96.33%
LSRCP/IDFG - McCall (SFSR)	920	909 (98.8%)	97.62%	946	929(98.20%)	96.44%	880	878 (99.8%)	99.55%
LSRCP/ODFW - Imnaha	241	240 (99.6%)	99.18%	226	223(98.67%)	97.36%	245	242 (98.8%)	97.57%
LSRCP/ODFW/NPT – Lostine *	109/255	106 (97.2%)	17.28%	106/370	105(99.06%)	4.9%	129/279	126 (97.7%)	20.40%
LSRCP/ODFW - Catherine Creek *	57/216	57 (100%)	6.96%	80/82	79 (98.75%)	23.8%	74/74	70 (94.6%)	89.48%
LSRCP/ODFW - Grande Ronde *	27/265	27 (100%)	1.04%	112/257	109(97.32%)	8.9%	155/155	154 (99.4%)	98.71%
LSRCP/ODFW - Lookingglass Creek	149	148 (99.3%)	98.66%	65	63(96.92%)	93.94%	155	154 (99.4%)	98.71%
Nez Perce Tribal Hatchery (NPTFH)	193	191 (98.96%)	97.94%	428	420(98.13%)	96.3%	491	487 (99.2%)	98.38%
Johnson Cr.	62	62 (100%)	100.00%	54	54 (100%)	100%	†	†	†
Total	9782	9686 (99.0%)	98.05%	8776	8349(95.14%)	90.51%	8290	8235(99.3%)	98.68%

Table 5. Ranked estimates of null allele frequencies for 53 loci from the combined steelhead 2010 PBT broodstock.

SNP Name	Freq of null allele	SNP Name	Freq of null allele
OMS00039	<0.001	OMS00132	0.018
Omy_99300202	0.001	Omy_116733349	0.018
Omy_109894185	0.006	Omy_128923433	0.018
Omy_BACB4324	0.007	Omy_bcAKala380rd	0.018
Omy_114315438	0.008	Omy_srp0937	0.019
Omy_Il1b198	0.008	OMS00064	0.02
OMS00105	0.009	OMS00072	0.021
Omy_129870756	0.01	OMS00090	0.021
Omy_arp630	0.01	Omy_ntl27	0.021
Omy_101993189	0.011	Omy_Il1b_028	0.022
Omy_102505102	0.011	Omy_gluR79	0.023
Omy_cox1221	0.011	OMS00024	0.024
Omy_g1282	0.011	Omy_aldB165	0.024
M09AAE082	0.012	Omy_105714265	0.025
Omy_110064419	0.012	Omy_rbm4b203	0.025
Omy_b1266	0.012	OMS00101	0.028
Omy_oxct85	0.012	Omy_u0954311	0.031
OMS00053	0.013	Omy_metA161	0.033
OMS00077	0.013	Omy_Ogo4212	0.036
Omy_108007193	0.015	Omy_105105448	0.04
Omy_crb106	0.016	M09AAJ163	0.042
OMY1011SNP	0.016	Omy_anp17	0.044
OMS00074	0.017	OMS00089	0.053
OMS00079	0.017	OMS00118	0.053
Omy_114587480	0.017	OMS00070	0.055
Omy_rapd167	0.017	Omy_vatf406	0.068
		Omy_113490159	0.075

Table 6. Ranked estimates of null allele frequencies for 46 loci from the combined steelhead 2011 PBT broodstock.

SNP Name	Freq of null allele	SNP Name	Freq of null allele
Omy_rapd167	<0.001	OMS00072	0.016
OMS00039	0.001	OMS00064	0.018
Omy_114315438	0.006	OMS00111	0.021
Omy_NaKATPa350	0.007	Omy_Il1b_028	0.021
OMS00058	0.009	Omy_U11_2b154	0.021
OMS00079	0.009	Omy_crb106	0.022
Omy_11138351	0.009	Omy_redd1410	0.022
OMS00071	0.01	OMS00179	0.024
OMS00078	0.01	OMS00068	0.025
Omy_110064419	0.01	Omy_129870756	0.026
Omy_Il1b198	0.01	Omy_cox1221	0.027
OMS00002	0.012	Omy_metA161	0.027
Omy_99300202	0.012	Omy_114587480	0.03
Omy_10780634	0.013	Omy_bcAKala380rd	0.03
Omy_BACB4324	0.013	Omy_u0954311	0.031
Omy_rbm4b203	0.013	OMS00101	0.032
OMS00024	0.014	M09AAJ163	0.041
OMS00089	0.014	Omy_Ogo4212	0.046
Omy_130524160	0.014	Omy_anp17	0.048
Omy_97660230	0.014	Omy_vatf406	0.068
Omy_IL6320	0.014	OMS00070	0.072
Omy_oxct85	0.014	OMS00118	0.074
OMY1011SNP	0.014	Omy_113490159	0.078

Table 7. Ranked estimates of null allele frequency for 58 loci from the combined Chinook 2008 PBT dataset.

SNP Name	Freq of null allele	SNP Name	Freq of null allele
Ots_NOD1	0.003	Ots_GTH2B550	0.013
Ots_ARNT	0.006	Ots_Prl2	0.013
Ots_nkef192	0.008	Ots_MHC1	0.013
Ots_u0717135	0.008	Ots_IGFI176	0.013
Ots_9490399R	0.009	Ots_mapK3309	0.014
Ots_TGFB	0.009	Ots_100884287	0.014
Ots_129458451	0.01	Ots_Thio	0.014
Ots_ppie245	0.01	Ots_NFYB147	0.014
Ots_mapKpr151	0.01	Ots_96500180	0.015
Ots_112419131	0.01	Ots_112876371	0.016
Ots_mybp85	0.01	Ots_OTDESMIN19SNP1	0.016
Ots_u21185	0.01	Ots_117432409	0.017
Ots_102801308	0.01	Ots_Est740	0.017
Ots_12875761R	0.011	Ots_S71	0.017
Ots_brp1664	0.011	Ots_112820284	0.017
Ots_110201363	0.011	Ots_hsc713488	0.018
Ots_11055164	0.011	Ots_113242216	0.019
Ots_124774477	0.011	Ots_P53	0.019
Ots_FGF6B_1	0.011	Ots_105407117	0.02
Ots_103122180	0.011	Ots_108820336	0.02
Ots_GDH81x	0.012	Ots_u675	0.021
Ots_105105613	0.012	Ots_TAPBP	0.021
Ots_115987325	0.012	Ots_11230143	0.021
Ots_vatf251	0.012	Ots_u0725325	0.022
Ots_OTSTF1SNP1	0.012	Ots_txnip321	0.025
Ots_102414395	0.012	Ots_101704143	0.026
Ots_u492	0.012	Ots_94857232R	0.032
Ots_pigh105	0.013	Ots_MHC2	0.041
Ots_unk526	0.013	Ots_OTALDBINT1SNP1	0.062

Table 8. Ranked estimates of null allele frequency for 72 loci from the combined Chinook 2009 PBT dataset.

SNP Name	Freq of null allele	SNP Name	Freq of null allele
Ots_NOD1	<0.001	Ots_u100275	0.01
Ots_9490399R	<0.001	Ots_110064383	0.01
Ots_105385421	<0.001	Ots_E2275	0.011
Ots_96899357R	<0.001	Ots_FGF6B_1	0.011
Ots_ARNT	<0.001	Ots_u492	0.011
Ots_AsnRS60	0.002	Ots_cox1241	0.012
Ots_OTSTF1SNP1	0.003	Ots_11230143	0.012
Ots_110201363	0.004	Ots_124774477	0.012
Ots_105132200	0.004	Ots_Prl2	0.013
Ots_102414395	0.004	Ots_u0725325	0.013
Ots_S71	0.004	Ots_108820336	0.014
Ots_113242216	0.005	Ots_112419131	0.014
Ots_unk526	0.005	Ots_112820284	0.014
Ots_109525816	0.005	Ots_112876371	0.015
Ots_103122180	0.005	Ots_110495380	0.015
Ots_115987325	0.006	Ots_mapK3309	0.016
Ots_Est740	0.006	Ots_TAPBP	0.016
Ots_HSP90B100	0.006	Ots_GDH81x	0.017
Ots_parp3286	0.006	Ots_NFYB147	0.017
Ots_pigh105	0.007	Ots_94857232R	0.018
Ots_CirpA	0.007	Ots_P53	0.018
Ots_GPH318	0.007	Ots_100884287	0.019
Ots_110689218	0.007	Ots_HMGB173	0.019
Ots_hsc713488	0.007	Ots_ppie245	0.02
Ots_vatf251	0.008	Ots_txnip321	0.021
Ots_Thio	0.008	Ots_OTDESMIN19SNP1	0.022
Ots_SWS1op182	0.009	Ots_redd1187	0.022
Ots_MHC1	0.009	Ots_u675	0.023
Ots_brp1664	0.01	Ots_pop596	0.023
Ots_IGFI176	0.023	Ots_TLR3	0.029
Ots_129458451	0.024	Ots_101704143	0.034
Ots_123921111	0.024	Ots_GCSH	0.035
Ots_CD592	0.025	Ots_96500180	0.035
Ots_117432409	0.025	Ots_MHC2	0.037
Ots_mybp85	0.025	Ots_TGFB	0.038
Ots_105407117	0.027	Ots_OTALDBINT1SNP1	0.075

Table 9. Ranked estimates of null allele frequency for 48 loci from the combined Chinook 2010 PBT dataset.

SNP Name	Freq of null allele	SNP Name	Freq of null allele
Ots_ppie245	<0.001	Ots_102801308	0.015
Ots_u0717135	0.001	Ots_NOD1	0.016
Ots_105385421	0.002	Ots_115987325	0.016
Ots_9490399R	0.006	Ots_unk526	0.017
Ots_96500180	0.006	Ots_mapK3309	0.017
Ots_94857232R	0.007	Ots_100884287	0.017
Ots_redd1187	0.007	Ots_P53	0.017
Ots_ARNT	0.008	Ots_u492	0.017
Ots_txnip321	0.008	Ots_129458451	0.018
Ots_110064383	0.009	Ots_MHC1	0.018
Ots_pigh105	0.01	Ots_112419131	0.018
Ots_hsc713488	0.01	Ots_mybp85	0.019
Ots_u21185	0.01	Ots_S71	0.023
Ots_112820284	0.01	Ots_101554407	0.024
Ots_GCSH	0.011	Ots_IGFI176	0.024
Ots_110201363	0.011	Ots_101704143	0.025
Ots_pop596	0.011	Ots_u0725325	0.028
Ots_110689218	0.011	Ots_GTH2B550	0.029
Ots_123921111	0.011	Ots_TAPBP	0.034
Ots_RAG3	0.012	Ots_MHC2	0.036
Ots_HSP90B100	0.012	Ots_105407117	0.039
Ots_vatf251	0.013	Ots_u675	0.04
Ots_TGFB	0.014	Ots_OTALDBINT1SNP1	0.053
Ots_TLR3	0.014	Ots_Ikaros250	0.12

Table 10. Results of comparisons between phenotypic sex and genetically determined sex using the modified sex-specific assay for steelhead (IDFG-OMY-SEX) from the 2010 broodstocks that were run with the new *"OmyY1_2SEXY"* marker.

	Total Samples	Missing Genetic Data	Total Successful Genotypes	Corresponding	Non-corresponding	Phenotypic males misidentified as female	Phenotypic females misidentified as male	Total phenotypic males	Total phenotypic females
Dworshak	1644	32 (1.9%)	1612 (98.1%)	1462 (90.7%)	150 (9.3%)	0 (0.0%)	150 (9.3%)	696 (42.3%)	948 (57.7%)
Squaw Cr.	45	0 (0.0%)	45 (100%)	45 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	20 (44.4%)	25 (55.6%)
Pahsimeroi	1102	25 (2.3%)	1077 (97.7%)	1047 (97.2%)	30 (2.8%)	0 (0.0%)	30 (2.8%)	551 (50.0%)	551 (50.0%)
Wallowa	500	1 (0.2%)	499 (99.8%)	496 (99.4%)	3 (0.6%)	0 (0.0%)	3 (0.6%)	251 (50.2%)	249 (49.8%)
Touchet R.	28	0 (0.0%)	28 (100%)	28 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	13 (46.4%)	15 (53.6%)
Tucannon R.	34	2 (5.9%)	32 (94.1%)	32 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	16 (47.1%)	18 (52.9%)
Total	3353	60 (1.8%)	3293 (98.2%)	3110 (94.4%)	183 (5.6%)	0 (0.0%)	183 (5.6%)	1547 (46.1%)	1806 (53.9%)

Table 11. Results of comparisons between phenotypic sex and genetically determined sex using the modified sex-specific assay for Chinook salmon (IDFG-OTS-SEX) from the 2010 broodstocks that were run with the new *"Ots_SEXY3-1"* marker.

	Total Samples	Missing Genetic Data	Total Successful Genotypes	Corresponding	Non-corresponding	Phenotypic males misidentified as female	Phenotypic females misidentified as male	Total phenotypic males	Total phenotypic females
Grand Ronde R.	155	9 (5.8%)	146 (94.2%)	146 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	73 (47.1%)	82 (52.9%)
Lostine R.	129	0 (0.0%)	129 (100%)	129 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	53 (41.1%)	76 (58.9%)
Total	284	9 (3.2%)	275 (96.8%)	275 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	126 (44.4%)	158 (55.6%)

Table 12. Average observed and expected heterozygosity of hatchery steelhead stocks for SY2010 and SY2011.

	2010				2011			
	Avg het (Obs)	**SD**	**Avg het (Exp)**	**SD**	**Avg het (Obs)**	**SD**	**Avg het (Exp)**	**SD**
Dworshak	0.395	0.095	0.394	0.095	0.395	0.097	0.395	0.095
Lyons Ferry	0.418	0.099	0.418	0.091	0.424	0.095	0.422	0.084
Grande Ronde	0.435	0.087	0.431	0.074	0.432	0.084	0.426	0.074
Touchet	0.457	0.138	0.431	0.093	0.425	0.113	0.422	0.090
Tucannon	0.431	0.116	0.430	0.086	0.453	0.112	0.429	0.084
Little Sheep Crk.	0.426	0.107	0.417	0.091	0.421	0.098	0.421	0.091
Oxbow	0.429	0.078	0.427	0.075	0.441	0.082	0.431	0.072
Pahsimeroi	0.429	0.078	0.427	0.076	0.440	0.074	0.431	0.071
Sawtooth	0.429	0.069	0.430	0.066	0.430	0.077	0.427	0.070
EFSR	0.415	0.095	0.415	0.078	0.421	0.089	0.423	0.078
Squaw Crk.	0.419	0.108	0.416	0.089	0.408	0.117	0.397	0.097
Wallowa	0.424	0.076	0.426	0.074	0.432	0.078	0.427	0.071

Table 13. Average observed and expected heterozygosity of hatchery Chinook stocks in 2008, 2009, and 2010. * not available at time of reporting.

	2008				2009				2010			
	Avg het (Obs)	SD	Avg het (Exp)	SD	Avg het (Obs)	SD	Avg het (Exp)	SD	Avg het (Obs)	SD	Avg het (Exp)	SD
Clearwater	0.348	0.132	0.342	0.125	0.346	0.130	0.341	0.126	0.345	0.128	0.341	0.124
Catherine Crk.	0.350	0.147	0.352	0.134	0.351	0.136	0.348	0.127	0.379	0.143	0.359	0.124
Dworshak	0.342	0.122	0.345	0.123	0.344	0.125	0.345	0.125	0.338	0.129	0.339	0.127
Grande Ronde	0.337	0.153	0.347	0.134	0.340	0.156	0.332	0.138	0.344	0.140	0.341	0.133
Imnaha	0.349	0.138	0.344	0.129	0.345	0.134	0.342	0.129	0.351	0.134	0.343	0.126
Johnson Crk	0.331	0.150	0.326	0.138	0.331	0.150	0.329	0.142	*	*	*	*
Lookingglass	0.359	0.133	0.357	0.121	0.345	0.129	0.352	0.125	0.357	0.129	0.351	0.120
Lostine	0.340	0.149	0.329	0.138	0.338	0.152	0.327	0.137	0.347	0.136	0.341	0.131
S. Fk. Salmon R.	0.333	0.133	0.334	0.133	0.330	0.139	0.328	0.136	0.328	0.137	0.330	0.135
Nez Perce FH	0.333	0.123	0.342	0.124	0.347	0.125	0.345	0.122	0.347	0.126	0.344	0.121
Pahsimeroi	0.331	0.135	0.329	0.133	0.337	0.140	0.333	0.135	0.332	0.128	0.333	0.127
Powell	0.335	0.134	0.335	0.134	0.323	0.126	0.341	0.126	0.344	0.137	0.344	0.132
Rapid River	0.340	0.127	0.339	0.127	0.341	0.131	0.338	0.128	0.340	0.133	0.338	0.129
Sawtooth	0.336	0.142	0.337	0.142	0.334	0.139	0.335	0.138	0.336	0.145	0.334	0.139
Tucannon	0.362	0.149	0.349	0.135	0.351	0.154	0.334	0.141	0.347	0.140	0.342	0.136

Table 14. Population structure (Fst) among steelhead hatchery stocks sampled in 2010. Asterisks (*) indicate that Fst values were significantly different from zero.

	Grande Ronde	**Dworshak**	**EFSR**	**Little Sheep**	**Lyons Ferry**	**Oxbow**	**Pahsimeroi**	**Sawtooth**	**Squaw Crk**	**Touchet**	**Tucannon**	**Wallow**
Grande Ronde	---	*	*	*	*	*	*	*	*	*	*	*
Dworshak	0.048	---	*	*	*	*	*	*	*	*	*	*
EFSR	0.030	0.036	---	*	*	*	*	*	*	*	*	*
Little Sheep	0.025	0.072	0.043	---	*	*	*	*	*	*	*	*
Lyons Ferry	0.024	0.052	0.035	0.030	---	*	*	*	*	*	*	*
Oxbow	0.018	0.049	0.020	0.027	0.024	---	*	*	*	*	*	*
Pahsimeroi	0.019	0.054	0.021	0.027	0.029	0.006	---	*	*	*	*	*
Sawtooth	0.016	0.050	0.019	0.033	0.026	0.006	0.007	---	*	*	*	*
Squaw Crk	0.028	0.019	0.025	0.054	0.037	0.029	0.030	0.028	---	*	*	*
Touchet	0.016	0.059	0.037	0.021	0.015	0.024	0.026	0.025	0.038	---	*	*
Tucannon	0.008	0.041	0.025	0.018	0.012	0.012	0.014	0.013	0.026	0.005	---	*
Wallow	0.001	0.048	0.032	0.027	0.022	0.019	0.020	0.016	0.029	0.019	0.009	---

Table 15. Population structure (Fst) among steelhead hatchery stocks sampled in 2011. Asterisks (*) indicate that Fst values were significantly different from zero.

	Grande Ronde	Dworshak	EFSR	Little Sheep	Lyons Ferry	Oxbow	Pahsimeroi	Sawtooth	Squaw Crk	Touchet	Tucannon	Wallow
Grande Ronde	---	*	*	*	*	*	*	*	*	*	*	*
Dworshak	0.047	---	*	*	*	*	*	*	*	*	*	*
EFSR	0.026	0.034	---	*	*	*	*	*	*	*	*	*
Little Sheep	0.026	0.067	0.033	---	*	*	*	*	*	*	*	*
Lyons Ferry	0.022	0.051	0.029	0.024	---	*	*	*	*	*	*	*
Oxbow	0.024	0.048	0.017	0.020	0.021	---	*	*	*	*	*	*
Pahsimeroi	0.024	0.053	0.015	0.021	0.023	0.004	---	*	*	*	*	*
Sawtooth	0.022	0.050	0.017	0.025	0.023	0.004	0.006	---	*	*	*	*
Squaw Crk	0.050	0.018	0.040	0.071	0.057	0.050	0.055	0.049	---	*	*	*
Touchet	0.018	0.043	0.023	0.017	0.012	0.017	0.018	0.022	0.048	---	*	*
Tucannon	0.013	0.043	0.016	0.017	0.009	0.010	0.011	0.013	0.045	<0.001	---	*
Wallow	0.007	0.048	0.022	0.022	0.020	0.016	0.017	0.015	0.047	0.017	0.009	---

Table 16. Population structure (Fst) among Chinook hatchery stocks sampled in 2008. Asterisks (*) indicate that Fst values were significantly different from zero (p <0.01).

	Clearwater	Catherine	Dworshak	Grande Ronde	Imnaha	Johnson	Looking.	Lostine	SF Sal.	NPFH	Pahsim.	Powell	Rapid	Sawtooth	Tucannon
Clearwater	---	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Catherine	0.023	---	*	*	*	*	*	*	*	*	*	*	*	*	*
Dworshak	0.005	0.021	---	*	*	*	*	*	*	*	*	*	*	*	*
Grande Ronde	0.011	0.021	0.012	---	*	*	*	*	*	*	*	*	*	*	*
Imnaha	0.012	0.023	0.012	0.010	---	*	*	*	*	*	*	*	*	*	*
Johnson Crk	0.021	0.027	0.018	0.022	0.019	---	*	*	*	*	*	*	*	*	*
Lookingglass	0.010	0.012	0.010	0.010	0.014	0.022	---	*	*	*	*	*	*	*	*
Lostine	0.024	0.037	0.025	0.020	0.021	0.044	0.028	---	*	*	*	*	*	*	*
SF Salmon	0.019	0.033	0.016	0.020	0.015	0.011	0.024	0.028	---	*	*	*	*	*	*
NP FH	0.002	0.021	0.001	0.011	0.012	0.018	0.008	0.025	0.016	---	*	*	*	*	*
Pahsimeroi	0.038	0.053	0.035	0.036	0.031	0.038	0.041	0.046	0.027	0.037	---	*	*	*	*
Powell	0.007	0.035	0.011	0.024	0.021	0.028	0.018	0.036	0.027	0.010	0.043	---	*	*	*
Rapid River	0.012	0.024	0.014	0.019	0.018	0.025	0.019	0.031	0.026	0.010	0.047	0.031	---	*	*
Sawtooth	0.029	0.045	0.028	0.029	0.033	0.028	0.035	0.043	0.020	0.030	0.024	0.034	0.037	---	*
Tucannon	0.032	0.036	0.031	0.028	0.033	0.041	0.033	0.045	0.039	0.032	0.057	0.040	0.047	0.055	---

Table 17. Population structure (Fst) among Chinook hatchery stocks sampled in 2009. Asterisks (*) indicate that Fst values were significantly different from zero (p <0.01).

	Clearwater	Catherine	Dworshak	Grande Ronde	Imnaha	Johnson	Looking.	Lostine	SF Sal.	NPFH	Pahsim.	Powell	Rapid	Sawtooth	Tucannon
Clearwater	---	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Catherine	0.018	---	*	*	*	*	*	*	*	*	*	*	*	*	*
Dworshak	0.012	0.014	---	*	*	*	*	*	*	*	*	*	*	*	*
Grande Ronde	0.016	0.025	0.015	---	*	*	*	*	*	*	*	*	*	*	*
Imnaha	0.014	0.014	0.014	0.025	---	*	*	*	*	*	*	*	*	*	*
Johnson Crk	0.025	0.023	0.022	0.034	0.021	---	*	*	*	*	*	*	*	*	*
Lookingglass	0.011	0.007	0.008	0.015	0.011	0.024	---	*	*	*	*	*	*	*	*
Lostine	0.024	0.022	0.021	0.028	0.016	0.041	0.019	---	*	*	*	*	*	*	*
S F Salmon	0.021	0.021	0.018	0.029	0.014	0.011	0.021	0.029	---	*	*	*	*	*	*
NP FH	0.009	0.012	0.0004	0.014	0.012	0.021	0.008	0.020	0.017	---	*	*	*	*	*
Pahsimeroi	0.039	0.029	0.032	0.041	0.028	0.036	0.031	0.036	0.030	0.032	---	*	*	*	*
Powell	0.009	0.016	0.005	0.016	0.014	0.024	0.012	0.022	0.02	0.005	0.037	---	*	*	*
Rapid River	0.003	0.022	0.02	0.021	0.018	0.032	0.016	0.030	0.029	0.016	0.044	0.015	---	*	*
Sawtooth	0.027	0.024	0.026	0.033	0.024	0.028	0.025	0.035	0.02	0.024	0.025	0.026	0.031	---	*
Tucannon	0.044	0.036	0.031	0.063	0.032	0.043	0.037	0.045	0.044	0.03	0.057	0.037	0.053	0.058	---

Table 18. Population structure (Fst) among Chinook hatchery stocks sampled in 2010. Asterisks (*) indicate that Fst values were significantly different from zero ($p < 0.01$).

	Clearwater	Catherine	Dworshak	Grande Ronde	Imnaha	Looking.	Lostine	Tucannon	NPFH	Pahsim.	Powell	Rapid	Sawtooth	SFSR
Clearwater	---	*	*	*	*	*	*	*	*	*	*	*	*	*
Catherine	0.011	---	*	*	*	*	*	*	*	*	*	*	*	*
Dworshak	0.006	0.013	---	*	*	*	*	*	*	*	*	*	*	*
Grande Ronde	0.019	0.019	0.022	---	*	*	*	*	*	*	*	*	*	*
Imnaha	0.016	0.013	0.017	0.025	---	*	*	*	*	*	*	*	*	*
Lookingglass	0.009	0.005	0.009	0.017	0.013	---	*	*	*	*	*	*	*	*
Lostine	0.031	0.023	0.034	0.037	0.023	0.024	---	*	*	*	*	*	*	*
Tucannon	0.032	0.031	0.029	0.042	0.029	0.029	0.043	---	*	*	*	*	*	*
NP FH	0.002	0.011	0.005	0.020	0.016	0.008	0.030	0.028	---	*	*	*	*	*
Pahsimeroi	0.039	0.037	0.036	0.040	0.033	0.036	0.044	0.055	0.037	---	*	*	*	*
Powell	0.004	0.013	0.004	0.022	0.016	0.009	0.033	0.029	0.003	0.036	---	*	*	*
Rapid River	0.013	0.015	0.022	0.026	0.020	0.015	0.036	0.045	0.017	0.048	0.019	---	*	*
Sawtooth	0.027	0.026	0.026	0.039	0.027	0.026	0.041	0.054	0.027	0.024	0.026	0.036	---	*
SF Salmon	0.018	0.020	0.020	0.028	0.016	0.018	0.037	0.040	0.017	0.035	0.019	0.031	0.021	---

Table 19. Proportion of individuals from the 2010 steelhead broodstocks that correctly assigned to their population of origin and the population to which the largest proportion misassigned.

	N	% Correct	Largest Misidentification and %	
Grande Ronde	80	33.8%	Wallowa	40.0%
Dworshak	1144	89.4%	Squaw Crk	5.2%
EFSR	109	74.3%	Dworshak	4.6%
Little Sheep Crk	52	76.9%	Grande Ronde	3.8%
Lyons Ferry	136	71.3%	Tucannon	6.6%
Oxbow	371	51.2%	Sawtooth	12.4%
Pahsimeroi	767	52.5%	Oxbow	13.3%
Sawtooth	758	49.9%	Pahsimeroi	14.5%
Squaw Crk	30	63.3%	Dworshak	13.3%
Touchet	11	27.3%	Tucannon	27.3%
Tucannon	21	23.8%	Lyons Ferry	14.3%
Wallowa	371	48.2%	Grande Ronde	21.3%

Table 20. Proportion of individuals from the 2011 steelhead broodstocks that correctly assigned to their population of origin and the population to which the largest proportion misassigned.

	N	% Correct	Largest Misidentification and %	
Grande Ronde	120	57.5%	Wallowa	14.2%
Dworshak	1400	86.5%	Squaw Crk	6.2%
EFSR	68	58.8%	Pahsimeroi	8.8%
Little Sheep Crk	96	72.9%	Grande Ronde	6.3%
Lyons Ferry	134	62.7%	Tucannon	8.2%
Oxbow	275	38.2%	Pahsimeroi	18.2%
Pahsimeroi	520	48.7%	Oxbow	15.4%
Sawtooth	616	50.6%	Oxbow	13.5%
Squaw Crk	48	54.2%	Dworshak	25.0%
Touchet	27	37.0%	Tucannon	25.9%
Tucannon	14	21.4%	EFSR	14.3%
Wallowa	335	53.4%	Grande Ronde	18.2%

Table 21. Proportion of individuals from the 2008 Chinook broodstock that correctly assigned to their population of origin and the population to which the largest proportion misassigned. (The program ONCOR removes samples with any missing data before analysis; thus, samples sizes for this analysis are lower than samples sizes reported in Table 4 and in the case of Nez Perce FH samples, all samples had missing data for at least 1 SNP and could not be analyzed)

	N	% Correct	Largest Misidentification and %	
Clearwater	681	25.0%	Powell	20.4%
Catherine Crk	53	58.5%	Imnaha	7.5%
Dworshak	960	30.2%	NPFH	11.5%
Grande Ronde	23	30.4%	Imnaha	13.0%
Imnaha	175	45.7%	Rapid	8.0%
Johnson Crk	55	47.3%	SF Salmon	18.2%
Lookingglass	109	46.8%	Imnaha	11.9%
Lostine	94	69.1%	Imnaha	7.4%
SF Salmon	1734	61.3%	Johnson Crk	7.8%
Nez Perce FH	0	N/A	N/A	N/A
Pahsimeroi	622	79.9%	SF Salmon	5.1%
Powell	166	68.1%	Clearwater	9.0%
Rapid	902	68.6%	NPFH	4.5%
Sawtooth	1099	73.4%	Pahsimeroi	7.2%
Tucannon	62	79.0%	Dworshak	3.2%

Table 22. Proportion of individuals from the 2009 Chinook broodstocks that correctly assigned to their population of origin and the population to which the largest proportion misassigned.

	N	% Correct	Largest Misidentification and %	
Clearwater	422	27.0%	Rapid River	32.7%
Catherine Crk	61	54.1%	Imnaha	8.2%
Dworshak	386	28.8%	NPFH	17.6%
Grande Ronde	98	62.2%	Lookingglass	8.2%
Imnaha	145	38.6%	Lostine	11.7%
Johnson Crk	48	58.3%	SF Salmon	18.8%
Lookingglass	59	28.8%	Catherine Crk	13.6%
Lostine	82	72.0%	Grande Ronde	6.1%
SF Salmon	560	62.0%	Sawtooth	7.5%
Nez Perce FH	380	18.2%	Dworshak	26.6%
Pahsimeroi	488	83.4%	Sawtooth	6.4%
Powell	349	32.7%	Rapid River	14.0%
Rapid	10	70.0%	Clearwater	10.0%
Sawtooth	798	72.9%	SF Salmon	5.4%
Tucannon	86	80.2%	Imnaha	5.8%

Table 23. Proportion of individuals from the 2010 Chinook broodstocks that correctly assigned to their population of origin and the population to which the largest proportion misassigned.

	N	% Correct	Largest Misidentification and %	
Clearwater	493	25.6%	Rapid	13.6%
Catherine Crk	64	39.1%	Lookingglass	15.6%
Dworshak	858	37.4%	Powell	11.7%
Grande Ronde	60	63.3%	Clearwater	5.0%
Imnaha	213	54.5%	Rapid	6.1%
Lookingglass	114	37.7%	Catherine Crk	10.5%
Lostine	114	79.8%	Imnaha	3.5%
Tucannon	118	79.7%	Imnaha	5.9%
Nez Perce FH	400	24.3%	Dworshak	13.8%
Pahsimeroi	479	80.6%	Sawtooth	5.4%
Powell	227	39.2%	Dworshak	12.3%
Rapid	1396	69.6%	Clearwater	7.0%
Sawtooth	510	75.5%	Pahsimeroi	5.7%
SF Salmon	851	65.9%	Sawtooth	6.1%

Table 24. Estimates of effective population size and 95% confidence intervals for steelhead hatchery stocks sampled in 2010 and 2011.

	2010		2011	
	Ne	95% CI	Ne	95% CI
Grande Ronde	184.3	143.4 - 251.4	83.6	75.6 - 92.7
Dworshak	313.2	293.1 - 334.7	220.2	208.2 - 232.8
EFSR	31.9	29.5 - 34.6	45.6	41.0 - 50.9
Little Sheep Crk.	130.9	107.4 - 164.4	254.3	193.1 - 361.2
Lyons Ferry	127.9	113 - 145.9	122.1	107.2 - 140.3
Oxbow	230.6	209.2 - 255.1	193.9	174.7 - 216.1
Pahsimeroi	270.1	251 - 291	218.7	203.7 - 234.9
Sawtooth	193.6	180.4 - 207.8	219.1	203.1 - 236.6
Squaw Crk	46.5	38.5 - 57.5	35.3	31.1 - 40.3
Touchet	120	65.5 - 506.8	702.5	174.7 – Inf.
Tucannon	139.6	81.5 - 404.9	83.9	59.4 - 135.1
Wallowa	209.1	190.2 - 230.5	188.0	171.6 - 206.5

Table 25. Estimates of effective population size and 95% confidence intervals for Chinook hatchery stocks sampled in 2008, 2009, and 2010. * Not available at time of reporting.

	2008		2009		2010	
	Ne	**95% CI**	**Ne**	**95% CI**	**Ne**	**95% CI**
Clearwater	267.9	247.9-289.7	238.5	218.5-260.9	214.8	198.0-233.3
Catherine Crk	38.9	33.6-45.5	62.7	54-73.9	108.1	85.6-143.1
Dworshak	438	400.8-479.7	434.4	396.6-476.9	276.8	257.6-297.5
Grande Ronde	67.8	44.3-130.8	47.3	42.5-53.0	80.2	71.1-90.9
Imnaha	256.7	216.7-310.3	317.2	256.2-408.1	237.3	201.6-284.4
Johnson Crk	2931.8	365.5-Inf	114.4	83.4-173.8	*	*
Lookingglass	81	72.2-91.3	176.2	122.2-299.1	120.7	104.6-141.0
Lostine	99.2	84.2-119.1	142.0	114.4-182.9	99.6	85.7-117.3
SF Salmon	358.7	335.2-384	415.0	366.8-473.2	361.2	330.4-395.7
Nez Perce FH	306.7	242.2-408.4	321.9	280.8-373.1	116.6	108.3-125.7
Pahsimeroi	142.8	132.8-153.6	165.9	153.1-179.9	146.5	135.4-158.7
Powell	147.3	137.6-157.6	126.2	117.9-135.2	202.8	182.1-226.8
Rapid	593.8	552.8-638.1	753.5	681.9-835.8	606.1	561.0-655.4
Sawtooth	132.6	124.7-141	181.9	168.9-195.9	138.2	127.9-149.4
Tucannon	137.1	113.7-169.7	158.5	133.7-191.6	114.1	99.4-132.5

Table 26. PBT assignment results for steelhead with CWTs collected in the Idaho fishery during 2010. Samples represent only individuals expected to assign the 2008 PBT database.

CWT-based origin	**Samples**	**Genotyped (Failed to amplify)**	**Assigned**	**Assignment Success**
Dworshak	9	9 (0)	7	77.8%
Pahsimeroi	27	27 (0)	27	100%
Oxbow	16	15 (1)	10	66.7%
Sawtooth	4	3 (1)	3	100%
Upper Salmon B	1	1 (0)	1	100%
Lyons Ferry – G.R.	1	1 (0)	1	100%
Mixed Upper Salmon A	3	3 (0)	3 (Pahsimeroi)	100%
Total	61	59 (2)	52	88.1%

Table 27. Sample sizes and hatchery of origin identified by coded wire tags (CWTs) for Chinook sampled in 2011 creel surveys. Number of samples expected to assign was determined as the number of age 3 fish using CWT data. * One individual identified as a 4-year-old via CWT data assigned to PBT parents.

CWT-based origin	Genotyped	Expected to Assign to 2008 PBT baseline	PBT Assigned	Assignment Success
Clearwater	69	27	22*	77.8%
Rapid River	45	18	16	88.9%
Sawtooth	15	14	14	100%
SF Salmon	7	6	6	100%
Dworshak	31	13	13	100%
Lookingglass	8	4	0	0%
Pahsimeroi	11	1	1	100%
Total	186	83	72	85.6%

Table 28. Number of returning adult strays to the Deschutes River in 2011 that assigned to two parents spawned as broodstock in 2008 at Snake River hatcheries.

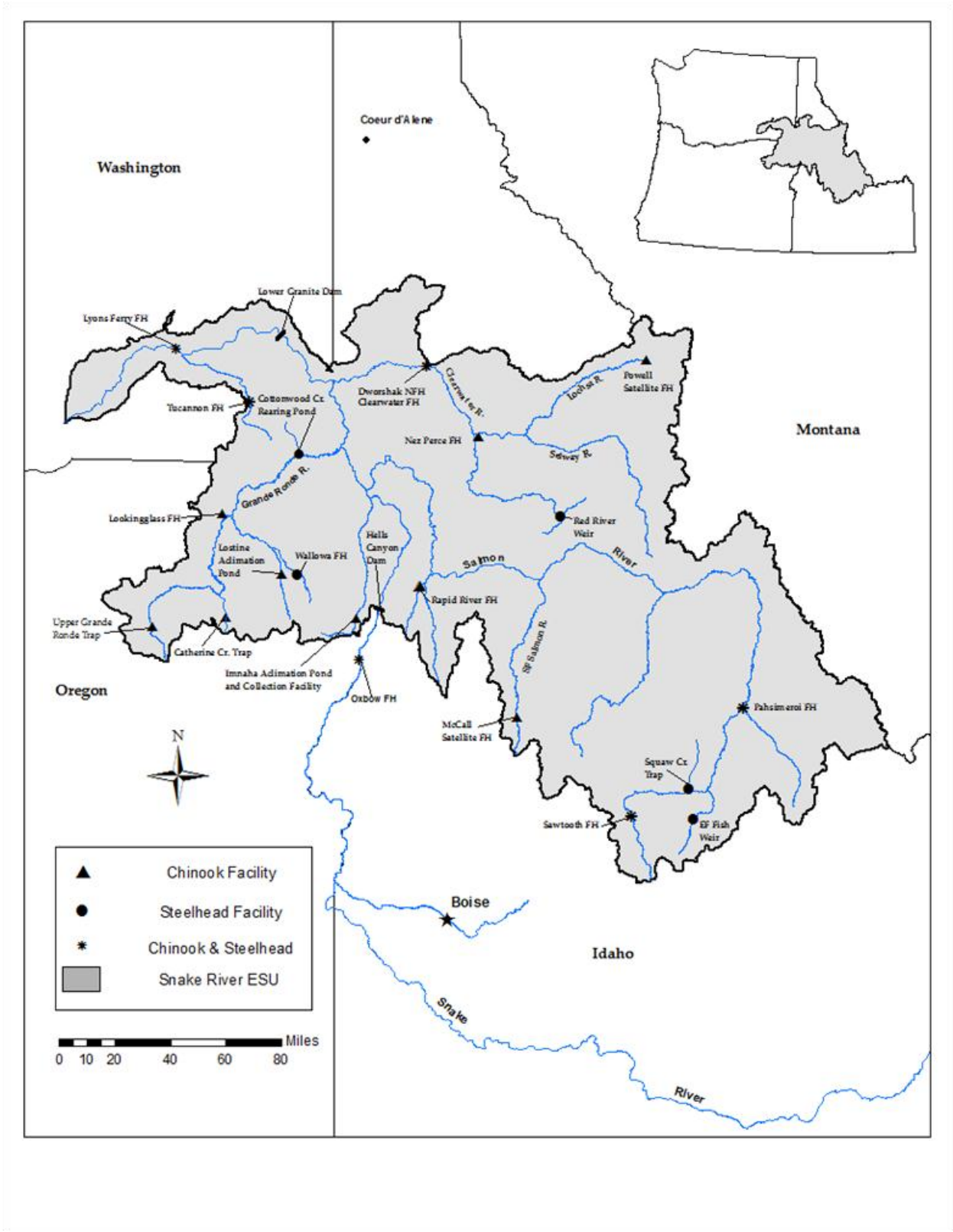
Snake River Parent Hatchery	Num. Strays
Dworshak	28
East Fork South Fork Salmon	1
Lyons Ferry, Grand Ronde	1
Oxbow	34
Pahsimeroi	33
Sawtooth	30
Squaw Creek	7

Table 29. PBT assignment results for ad-clipped hatchery kelts collected at Lower Granite Dam

Origin	Brood year	Number (Prop.)
Grande Ronde	2008	14 (8.4%)
Dworshak	2008	5 (3.0%)
Pahsimeroi	2008	61 (36.8%)
Sawtooth	2008	71 (42.8%)
Oxbow	2008	14 (8.4%)
Sawtooth	2009	1 (0.6%)

FIGURES

Figure 1. Location of sampled fish hatcheries in the Snake River basin.



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